Original Article

Vitamin D levels and microvascular complications in type 2 diabetes

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ABSTRACT

Background: Vitamin D has important actions on glucose metabolism. These include improved insulin exocytosis, direct stimulation of insulin receptor, improved uptake of glucose by peripheral tissues, improving insulin resistance. It has got various pleiotropic effects like suppression of cell mediated immunity, regulation of cell proliferation, stimulation of neurotropic factors such as nerve growth factor, Glial cell line-derived neurotrophic factor, neurotropin, suppression of RAAS, reduction of albuminuria, immunomodulatory effects, and anti-inflammatory effects. Thus, vitamin D is implicated in many ways in the pathogenesis of retinopathy, neuropathy and nephropathy. **Objectives:** To study the correlation of vitamin D levels with microvascular complications in type 2 diabetes. **Materials and Methods:** Cross-sectional case-control study of 18 patients (18-70 years), who met the American Diabetes Association 2011 criteria for type 2 diabetes, was conducted. Age and sex matched healthy controls were taken. Subjects were evaluated for the presence of microvascular complications by clinical evaluation, urine examination, fundus examination, nerve conduction studies, and various biochemical tests. 25-OH cholecalciferol levels were done for each. Cut off level for vitamin D deficiency was 20 ng/ml. **Results:** Mean vitamin D was lower in type 2 diabetics than healthy subjects (19.046 vs. 27.186 ng/ml). Prevalence of vitamin D deficiency and insufficiency was found to significantly higher in diabetics when compared to healthy subjects ($P = 0.0001$). Vitamin D deficiency was found to be significantly associated with neuropathy ($\chi^2 = 5.39$, df = 1, $P = 0.020$), retinopathy, ($\chi^2 = 6.6$, df = 1, $P = 0.010$) and nephropathy ($\chi^2 = 10.52$, df = 1, $P = 0.001$). Lower levels of vitamin D were found to be associated with increasing prevalence of combinations of microvascular complications namely neuropathy with retinopathy ($P = 0.036$), neuropathy with nephropathy ($P = 0.029$), retinopathy with nephropathy ($P = 0.022$) and neuropathy with retinopathy with nephropathy ($P = 0.0001$).

Key words: Diabetes, nephropathy, neuropathy, retinopathy, vitamin D

Introduction

Vitamin D has important actions on glucose metabolism. These include improved insulin exocytosis, direct stimulation of insulin receptor, improved uptake of glucose by peripheral tissues, improving insulin resistance.[1-3] It has got various pleiotropic effects such as suppression of cell mediated immunity, regulation of cell proliferation, stimulation of neurotropic factors like nerve growth factor, Glial cell line-derived neurotrophic factor, neurotropin, suppression of RAAS, reduction of albuminuria, immunomodulatory, anti-inflammatory and antiangiogenic effects.[4-12] Thus vitamin D is implicated in many ways in the pathogenesis of diabetic retinopathy, neuropathy and nephropathy.

This study was carried out to study vitamin D levels in type 2 diabetics and evaluate the association of vitamin D levels with microvascular complications in type 2 diabetes.

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Materials and Methods

The present study is an observational single centre case-control study. Ethical committee clearance was duly taken. Cases comprised of persons with type 2 diabetes aged 18-70 years with or without microvascular complications who were not receiving vitamin D or calcium

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supplementation. The control group comprised of age, sex and socioeconomically matched normal healthy volunteers. Informed consent was taken from all the samples included in the study.

Subjects with type 1 diabetes mellitus, glycosylated hemoglobin (HbA1c) ≥7.5%, vitamin D intake greater than 1000 IU/day, serum calcium <8 or >11 mg/dL, creatinine >1.5 mg/dL, white blood cell <2,000 or >15,000/mm^3, urine albumin to creatinine ratio >150 were excluded. Patients having disorders that change the metabolism of vitamin D, significant cardiac, hepatic, renal and oncologic disease, use of medications known to affect serum phosphate levels, calcitonin, calcitriol, growth hormone, anticonvulsants, hormone replacement therapy, steroids, testosterone or vitamin A (>20,000 units/day) were also excluded. Those having sun exposure less than 3 h/week were also excluded. The screening was done in each case to assess the associated microvascular complications, which include complete physical examination, microfilament test, nerve conduction velocity, detailed fundus examination, ultrasonography of the abdomen and other biochemical investigations. fasting plasma glucose, 2 h postprandial blood sugar, HbA1c, serum vitamin D levels (25-OH vitamin D), calcium, phosphorus, urea, creatinine, liver function test and lipid profile, complete blood count, thyroid stimulating hormone, urine routine microscopy, urine microalbumin by creatinine ratio, electrocardiogram and chest X-ray were done in all subjects under study. Vitamin D deficiency was defined as levels <20 ng/ml and insufficiency 20-29 ng/ml in accordance to WHO definition.[13] Diabetic nephropathy was defined by spot urine albumin by creatinine ratio of >30. Since, vitamin D levels are affected in later stages of chronic kidney disease thus diabetics with urine albumin by creatinine ratio >150 were excluded. Vitamin D levels were done from a single laboratory using same lab assay. Chi-square test, Student's *t*-test and ANOVA test were used to study the statistical significance of data obtained.

Results

A total of 158 cases were studied. 130 age and sex matched healthy volunteers served as controls. The mean age of cases under study was 52.85 ± 8.26 years compared to 51.87 ± 6.43 years of controls. 60.12% were males, whereas 39.88% were females in the case group while in the control group, 61.25% were males, whereas 38.75% were females. The mean duration of diabetes in the cases studied was 5.34 ± 3.09 years. It was 3.84 ± 2.7 years in cases having neuropathy alone and 3.88 ± 2.7 years and 3.88 ± 1.9 years respectively in cases having nephropathy and retinopathy alone. In cases having two microvascular complications the mean duration of diabetes was 4.3 ± 1.34 years (neuropathy with retinopathy), 4.63 ± 2.9 years (neuropathy with nephropathy) and 5.33 ± 3.24 years (retinopathy with nephropathy). Vitamin D deficiency is now a worldwide recognized problem. Most of the natural foods are deficient in vitamin D and fortification is also inadequate.[17] In the cases having all the three of neuropathy, retinopathy and nephropathy the mean age was 8 ± 2.84 years.

The mean vitamin D level was 19.046 ± 6.614 ng/ml in diabetics, while it was 27.186 ± 9.361 ng/ml in the control group. Out of the total 158 cases (59.49%) had vitamin D <20 ng/ml and only 10 (6.33%) had above 30 ng/ml. On the contrary in the control group, 45 had vitamin D level <20 ng/ml and 45 (34.61%) had levels more than 30 ng/ml. There was a significant difference in levels of vitamin D in diabetics and non-diabetics (t = 8.624, df = 286, P = 0.0001). The mean vitamin D was found to be 19.43 ± 6.418 in males and 18.56 ± 5.916 in females in diabetics, and the difference between two genders was found to be insignificant (P = 0.5651). Similarly, no significant difference was found in the vitamin D levels in males and females in the control group as well.

Vitamin D deficiency (<20 ng/ml) was present in 59.49% of the cases and 34.61% of controls. Only 6.33% cases had vitamin D level >30 ng/ml, while 35.39% of controls were found to be have sufficient (>30 ng/ml) levels of vitamin D. Overall vitamin D was found to be inadequate (vitamin D deficiency with insufficiency, or in other words levels <30 ng/ml) in 93.67% of cases and 64.61% of non-diabetics (controls). Prevalence of vitamin D deficiency and insufficiency was found to significantly higher in diabetics as compared to healthy subjects (χ^2 = 36.61, df = 1, P = 0.0001). Overall vitamin D deficiency was found to be more prevalent in diabetics (χ^2 = 16.7, df = 1, P = 0.0001).

Out of the 158 type 2 diabetics studied 41 (25.95%) had no microvascular complication. Single microvascular complications (retinopathy or neuropathy or nephropathy) was present in 29.11% of cases, while the combination of two was present in 25.32% and all three in 19.62% of cases.

In total, 56 cases had neuropathy (either singly or in combination with neuropathy or retinopathy). Out of these, 43 were vitamin D deficient. Similarly, 43 out of 54 having retinopathy and 44 out 54 having nephropathy were found to be having vitamin D deficiency. The percentage prevalence of vitamin D deficiency in various groups is given in Table 1. Vitamin D deficiency was found to be significantly associated with neuropathy (χ^2 = 5.39, df = 1, P = 0.020), retinopathy, (χ^2 = 6.6, df = 1, P = 0.010) and nephropathy (χ^2 = 10.5, df = 1, P = 0.001).

On evaluating various combinations of microvascualar complications, it was found that in cases having only one microvascular complication 46.15%, 52.38%, and 55.5% of those having neuropathy, retinopathy, and nephropathy respectively were found deficient for vitamin D. In cases having more than one microvascular complication, the percentage of vitamin D deficiency further rose to 75% (neuropathy with retinopathy), 77.77% (neuropathy with nephropathy) and 80% (retinopathy with nephropathy). In cases who had all the three of neuropathy, retinopathy and nephropathy vitamin D deficiency was92.3%. Among type 2 diabetics without any microvascular complication 51.22% were deficient in vitamin D.

The prevalence of vitamin D deficiency and mean vitamin D levels in various combinations of microvascular complications is given in Table 2. With an increasing number of complications the mean vitamin D levels were found to fall while the prevalence of vitamin D deficiency increased accordingly. The decline in vitamin D levels between the groups was found to be significant ($P = 0.001$ ANOVA). Further when individual groups were tested for the difference in vitamin D levels against the group having no microvasclar complication it was found that difference in levels of vitamin D in neuropathy, nephropathy and retinopathy as a single occurrence was insignificant ($P = 0.263$, 0.126, and 0.292 respectively). On the other hand, the difference was found to be significant in cases of neuropathy with retinopathy ($P = 0.036$), neuropathy with nephropathy ($P = 0.029$), retinopathy with nephropathy ($P = 0.022$) and neuropathy with retinopathy with nephropathy ($P = 0.0001$). Thus, declining levels of vitamin D levels were significantly related to the presence of combinations of microvascular complications.

Table 1: Prevalence of vitamin D deficiency in patients with microvascular complications

Microvascular complication	Percentage prevalence of vitamin D deficiency	*P* value χ^2 test
None	51.22	
Neuropathy	76.78	0.024
Retinopathy	79.63	0.010
Nephropathy	81.48	0.001

Table 2: Mean vitamin D levels in various microvascular complications

Microvascular complication	Percentage prevalence of deficiency	Mean vitamin D level (ng/ml)	*P* value (*t* test)
None	51.22	23.10±6.12	
Neuropathy alone	46.15	19.94±5.21	0.263
Retinopathy alone	50	19.25±7.86	0.126
Nephropathy alone	55.5	19.82±5.05	0.292
Neuropathy+retinopathy	75	16.64±6.21	0.036
Neuropathy+nephropathy	77.77	17.12±4.46	0.029
Retinopathy+nephropathy	80	16.96±4.85	0.022
Neuropathy+retinopathy+ nephropathy	92.3	14.63±4.43	0.0001

Discussion

Low vitamin D levels have been demonstrated to predict the development of microvascular complications in diabetes.

Scragg *et al*. and Suzuki *et al*. in their observational study in type 2 diabetes mellitus (T2DM) subjects concluded that mean vitamin D level concentration in men were significantly higher than women.[14,15] However in our study, 59.15% of males and 60.12% of females in the case group and 33.27% of males and 35.46% of females in the control group were found to be deficient in vitamin D. No significant difference was found in vitamin D levels between the two genders in the case as well as a control group. Various studies have found an inverse relation between vitamin D levels and diabetes prevalence. Pittas *et al*. found that among women higher levels of vitamin D were associated with a lower risk for T2DM.[16] Liu *et al*. reported that individuals in the highest tertile of vitamin D levels have a 40% lower incidence of T2DM.[18] Gagnon *et al*. showed that during the 5-year follow-up of subjects, who developed diabetes had lower vitamin D levels compared with those who remained free of diabetes.[19] Pietschmann *et al*. and Isaia *et al*. showed in their respective studies that an association exists between low circulating concentrations of vitamin D and the prevalence of diabetes and impaired glucose tolerance.[20,21]

In this study, it was found that individuals with type 2 diabetes were having lower vitamin D than non-diabetics. The mean vitamin D was 19.046 ng/ml in diabetics, while it was 27.186 ng/ml in non-diabetics. The difference was significant ($P = 0.0001$). Vitamin D deficiency was significantly higher in cases (59.49%) as compared to controls (34.61%) ($P = 0.0001$). Overall prevalence of inadequacy of vitamin D (vitamin D deficiency and insufficiency, or in other words levels <30 ng/ml) was significantly higher (93.67%) in cases than in non-diabetics (64.61%) ($P = 0.0001$). Hence, this suggests that hypovitaminosis D is more prevalent in diabetics and the vitamin D levels are significantly lower in diabetics as compared to non-diabetics. These results match with those obtained by others like Payne *et al*. and Aksoy *et al*., who their study described that diabetic subjects had lower vitamin D levels than non-diabetic subjects.[22,23] However, Suzuki *et al*. in their observational study in T2DM subjects concluded that mean vitamin D level concentration in T2DM patients was not statistically different from normal. Different results may be due to smaller sample size, geographical location and the effect of weather.[15]

The prevalence of microvascular complications in our study was found to be 74.05%. Only 25.95% of diabetics were free of all microvascular complication. Single microvascular complications (retinopathy or neuropathy or nephropathy) was present in 29.11% of cases while the combination of two was present in 25.32% and all three in 19.62% of cases. Overall 76.78% of cases having neuropathy, 79.63% of cases having retinopathy and 81.48% of cases having nephropathy were deficient in vitamin D. Among diabetics having no microvascular complications, 51.22% of cases were having vitamin D deficiency. On applying Chi-square test vitamin D deficiency was found to be separately associated significantly with neuropathy ($P = 0.020$), retinopathy ($P = 0.010$) and nephropathy ($P = 0.001$) individually. The mean vitamin D levels were found to be 23.10 ± 6.12 (no microvascular complication), 19.94 ± 5.21 (neuropathy), 19.25 ± 7.86 (retinopathy), 19.82 ± 5.05 (nephropathy), 16.64 ± 6.21 (neuropathy with retinopathy), 17.12 ± 4.46 (neuropathy with nephropathy), 16.96 ± 4.85 (retinopathy with nephropathy) and 14.63 ± 4.43 ng/ml (neuropathy with retinopathy with nephropathy). This decrease in level of vitamin D was associated significantly with the presence of multiple microvascular complications (ANOVA, $P = 0.0001$).

There was a significant difference in the vitamin D levels of classes having no microvascular complication and those having multiple microvascular complications, i.e. neuropathy with retinopathy ($P = 0.036$), neuropathy with nephropathy ($P = 0.029$), retinopathy with nephropathy ($P = 0.022$) and neuropathy with retinopathy with nephropathy ($P = 0.0001$). Thus, lower vitamin D levels were found to be significantly associated with increased chances of having multiple microvascular complications.

Robinson *et al*. in his study found that vitamin D were significantly lower in those diabetics who had microvascular complications.[24] Aksoy *et al*. also showed that the mean vitamin D3 concentrations fell with increasing severity of diabetic retinopathy.[23] Payne *et al*. demonstrated that patients with DR were deficient in vitamin D and that diabetic subjects, especially those with proliferative diabetic retinopathy (PDR).[22] Suzuki *et al*. showed that the existence of PDR was significantly associated with a decrease in serum vitamin D concentrations.[15] Even in a study on type 1 diabetes, Kaur *et al*. found that retinopathy prevalence was higher in cases with vitamin D deficiency versus sufficiency.[25] Chaychi *et al*. in his study found that patients with diabetic polyneuropathy had a lower mean serum vitamin D level.[26] Soderstorm *et al*. demonstrated vitamin D insufficiency is associated with the adjusted composite measure of neuropathy.[27] Lee and Chen in their study on use of vitamin D as analgesic for neuropathic pain found that all patients were vitamin D insufficient and mean vitamin D level was 18 ng/ml.[28] Diaz *et al*. in their study found that 30.7% of adults with diabetes have nephropathy, 48.9% have vitamin D deficiency and 36.6% have vitamin D insufficiency.[29] Kim *et al*. in their study found that mean vitamin D level was 18.4 ± 9.8 in diabetic nephropathy and 86% of subjects were vitamin D insufficient and 46% were deficient.[30] Oh *et al*. found that in early stage 3 CKD mean vitamin D level was 20.4 ng/ml and 29.9% were deficient in vitamin D.[31] The results obtained in our study compare well with those obtained in above studies. Thus in conclusion, mean vitamin D levels are significantly lower in type 2 diabetics, vitamin D deficiency (<20 ng/ml) in type 2 diabetes is significantly associated with any of the individual microvascular complications, i.e. neuropathy, retinopathy, and nephropathy and type 2 diabetics with decreasing vitamin D levels have significantly increasing prevalence of combination of microvascular complications.

Limitations

The cross-sectional design of this study limits the ability to assess causality. Secondly, only one time point was recorded for the subjects in this study. There is possible selection bias as this is not a study of consecutive patients seen at our institution. Due to the small sample size results cannot be generalized.

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